500

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : A61K 49/00, 49/02, 49/04

A1

(11) International Publication Number: WO 85/ 02772

(43) International Publication Date: 4 July 1985 (04.07.85)

(21) International Application Number: PCT/SE84/00437

(22) International Filing Date: 20 December 1984 (20.12.84)

(31) Priority Application Number: 8307060-7

(32) Priority Date: 21 December 1983 (21.12.83)

(33) Priority Country: SE

(71)(72) Applicants and Inventors: SCHRÖDER, Ulf [SE/SE]; Ferievägen 9, S-223 67 Lund (SE). SALFORD, Leif, G. [SE/SE]; Finngatan 9, S-223 62 Lund (SE).

(74) Agent: WIKLUND, Ingrid; Awapatent AB, Box 5117, S-200 71 Malmö (SE).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), US.

Published
With international search report.
In English translation (filed in Swedish).

(54) Title: DIAGNOSTIC AND CONTRAST AGENT

(57) Abstract

Spheres or particles in which a diagnostic or contrast agent for use in, for example, NMR technique, magnetometry, ultrasonics or radiology has been enclosed and in which the matrix is built up of carbohydrates, polyamino acids or synthetic polymers.

DIAGNOSTIC AND CONTRAST AGENT

BACKGROUND

The invention relates to response particles, preferably spheres, and their use as a diagnostic and contrast agent.

In diagnostic medicine, contrast agents are today being used primarily in X-ray diagnostics where an increased contrast effect is obtained during examination of, for example, internal organs, such as the kidneys, the urinary tract, the digestive tract, the vascular system of the heart (angiography), etc. This contrast effect is based upon the fact that the contrast agent itself is less permeable to X-rays than the surrounding tissue, as a result of which a different blackening of the X-ray plate is obtained.

X-raying implies certain radiation hazards, but during angiography the complication risk is associated in particular with the use of contrast agents.

In recent years, a number of new methods have been introduced in diagnostic radiology. One such method goes by the name NMR (Nuclear Magnetic Resonance) which provides information not only about the distribution of the water content in a specific tissue (in contradistinction to radiology which merely gives a measure of the transmissibility of the X-rays in a specific tissue), but also about the chemical tissue structure which is different in normal and pathological tissue.

In the NMR method, a strong and homogeneous magnetic field is applied across the tissue to be examined. By studying the so-called relaxation times of the protons of the molecules present, especially the protons of the water, it is possible to produce, via comprehensive and complex computer calculations, a visual image of the structure of the tissue concerned.

There is, however, an interest in being able to

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GA	Gabon	MR	Mauritania
AU	Australia	GB	United Kingdom	MW	Malawi
BB	Barbados	HU	Hungary	NL	Netherlands
BE	Belgium	IT	Italy	NO	Norway
BG	Bulgaria	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali		
FR	France				

make a differential diagnosis between pieces of tissue having a high density of blood vessels and, alternatively, tissue having a low density of vessels. Such a situation which has considerable clinical interest, comprises the localisation of tumours which, in their periphery, have a higher density of vessels as compared with normal tissue.

One useful method in the context is to inject into the vascular system some type of particles responsive to a magnetic field and showing changes in the above-mentioned relaxation times.

These magnetically responsive particles interfere with the above-mentioned homogeneous magnetic field in that there is formed, around each individual particle, a field gradient which in its turn changes the relaxation times.

Put more simply, this means that "black holes" are formed around each particle which may be visualised and thus give an impression of the vessel density in the tissue in question.

In another diagnostic method, use may be made of the movability of magnetically responsive particles in a tissue. The basic principle of this method may be studied according to the following: If magnetically responsive particle are introduced into a magnetic field, the particles will align themselves in the direction of the field lines. If the field is removed or shut down, the magnetically responsive particles will change their position in response to processes in the tissue. The duration of this change may, however, vary between different tissues and also in dependence upon the localisation of the particles within the tissue in question. This variability of the response of the magnetic material may be utilised diagnostically. If magnetically responsive particles are administered to a patient, the distribution of the particles in different organs can be determined by means of a sensitive magnetometer capable of detecting

the above-mentioned changes (Nature (1983) 302, 336).

Ultrasonics is another visualisation technique in which sound-waves are reflected differently against different types of tissue, depending upon the acoustic impedance of these tissues. Also in this respect, there is an interest in being able to use some type of contrast agent in order to obtain an amplification of specific organs. Particles of different types have here been shown to provide changed echo effects and a changed resolution associated therewith (J. Acoust.Soc.Am. (1983) 74, 1006).

It is also possible to use magnetically responsive particles having a Curie point of about 42°C for use at hyperthermia. In this instance, the magnetically responsive particles are retained during the treatment of the hyperthermia by a magnetic field, but the moment the tissue temperature exceeds the Curie point, the particles disappear from the tissue because the magnetic responsiveness disappears at this temperature.

By labelling the particles with some gamma-radiating nuclide (for example technetium-99m) it is possible to localise the particles by means of a gamma camera and thereby also to combine the examination with some of the other techniques referred to above.

When using particles within any of the above-mentioned ranges, it is desired, in order to achieve optimal conditions, to be able to vary the amount of magnetically or otherwise responsive material, without affecting on that account the pharmacodynamic and circulatory characteristics of the particles. To be able to do this, one must use a technique which implies enclosing the responsive material in a matrix, preferably a matrix of spherical shape, and the matrix should per se satisfy the following criteria:

- biocompatible
- biologically degradable
- nonimmunogenic.

A matrix of this type normally is built up of some type of polymers which are held together by chemical bonds. Different types of polymers are available for making such matrices. However, the selection of polymers will be very limited if the above-mentioned criteria of the matrix are to be fulfilled.

One type of polymers that has proved useful in these contexts are the carbohydrates, especially those who are included in the body as a natural constituent. Endogenous carbohydrates are represented by starch and glycogen, but also dextran may be included in this group because of its prolonged use as a blood substituent in medical service.

The production of a carbohydrate matrix satisfying these criteria is described in PCT/SE82/00381, PCT/SE83/00106 and PCT/SE83/00268.

Another type of polymers that have proved to satisfy the said criteria are polyamino acids, such as proteins of the type albumin. The production of polyamino acid matrices is disclosed in US-PS 4,247,406.

Further types of polymers are represented by synthetic polymers, such as acrylates, polystyrene etc. The production of matrices from synthetic polymers is well documented in literature.

It is in this connection extremely advantageous if covalent cross-linking of the polymers can be avoided in the production of a useful matrix. For example, covalently cross-linked carbohydrate matrices have been found to produce transformed cells, in the form of granuloma, when used on humans (Am.Surg. (1955) 142, 1045).

When using covalently cross-linked proteins, there is a risk of immunological reactions because the resulting derivatised protein is not recognised by the body's immunity system as a protein belonging to the body. There are, however, for specific systems no alternatives to the covalent cross-linking, especially when using synthetic polymers or combinations of different polymers and cross-

linking agents in order to obtain a useful system. As an example, it is possible to cross-link acrylic polymers with starch and, alternatively, to cross-link starch with acrylates.

Another useful possibility which is described in literature is the production of larger particles from smaller particles. For example, it is possible to produce, from 0.5 μm particles, conglomerates of larger particles, for example in the range of about 10 μm.

Another factor of importance to the injection of spheres into the vascular system is that the spheres have a size that prevents them from getting stuck in the capillary system of different tissues during the first passage. To prevent this, the particle must have a diameter below 1 μm (Chem.Pharm.Bull. (1975) 23, 1452) and a surface structure of hydrophilic character.

When particles are injected into the vascular system, all particles will have collected after a given period of time in the liver or spleen (the RES system) because the normal function of these organs is to purify the blood of foreign particles. At present, there is only one method described which is capable of collecting particles to organs other than those mentioned above, and this is by utilising magnetically responsive particles or spheres.

This is of particular interest in the context of this invention because spheres containing magnetically responsive substances can be used and be made to stop in different tissues by means of the outer magnetic field. When the magnetically responsive particles then are stuck in the desired tissue, the tissue in question can simply be visualised by means of the NMR method or some of the other techniques referred to above.

DESCRIPTION OF THE INVENTION

The invention relates to responsive particles, preferably spheres, and their use as a diagnostic and

contrast agent. The invention shows that it is possible to utilise spheres as contrast agents, the responsive material being enclosed within a matrix. The responsive material may consist of, for example, magnetite particles enclosed in the form of discrete particles of varying size, or in the form of complexed ions.

One conceivable matrix for use in the context of this invention consists of carbohydrates that have been stabilised by crystallization, which means that the type of chemical bonds holding the polymeric network together is not covalent in character, mainly hydrogen bonds, van der Waals forces or, in some cases, ion bonds.

As carbohydrate, use may be made of all conceivable variants, including carbohydrates of varying molecular weight and/or substituted or otherwise derivatised carbohydrates. For example, it may be mentioned that it is possible to produce and use magnetically responsive carbohydrate particles in which the carbohydrate is of starch origin and low-molecular of the type glucose, maltose, dextrins etc., and successively increasing molecular weight up to native potato starch having a molecular weight of several millions. The same molecular weight range is applicable to other carbohydrate groups, such as dextran or glycogen.

Another matrix for use in the complex of this invention may consist of polyamino acids, such as the protein albumin in which the matrix is stabilised by heating and the cohering forces are not covalent in character, of the type hydrophobic interactions, hydrogen bonds, van der Waals forces or ion bonds. In a manner similar to what has been stated above, it is also possible to use synthetic polymers or combinations as matrix.

The following Example should not be regarded as restrictive and merely serves to illustrate the main features of the invention.

EXAMPLE

Dextran spheres having a size of about 1 μm with

enclosed magnetite particles (size 10-20 nm) were suspended in human serum. The relaxation times of the solution were measured with an NMR apparatus (Praxis II, Alnor Instrument AB, Nyköping) and compared with the relaxation times for the same serum without magnetically responsive dextran spheres. The following values of T1 and T2, respectively, were obtained.

		T1 (ms)	T2 (ms)
Serum without particles:		1660	400
Serum with particles: conc.:	0.05 mg/ml	1342	109
	0.1 mg/ml	1306	82.2
	0.2 mg/ml	1147	52.6
	0.5 mg/ml	968	30.7
	1.0 mg/ml	813	24.0
	2.0 mg/ml	688	19.9
	4.0 mg/ml	691	22.9

CLAIMS

1. A sphere or particle for use in diagnostics or as a contrast agent, c h a r a c t e r i s e d in that it consists of a matrix and a diagnostic and/or contrast agent enclosed within said matrix.

2. A sphere or particle as claimed in claim 1, c h a r a c t e r i s e d in that the diagnostic or contrast agent is magnetically responsive.

3. A sphere or particle as claimed in claim 1, c h a r a c t e r i s e d in that the diagnostic or contrast agent is X-ray impermeable.

4. A sphere or particle as claimed in claim 1, c h a r a c t e r i s e d in that the diagnostic or contrast agent reflects sound waves.

5. A sphere or particle as claimed in claim 1, c h a r a c t e r i s e d in that the contrast agent is a radioactive nuclide.

6. A sphere or particle as claimed in one or more of claims 1-5, c h a r a c t e r i s e d in that the matrix has been stabilised by means of non-covalent bonds.

7. A sphere or particle as claimed in one or more of claims 1-6, c h a r a c t e r i s e d in that the matrix material consists of carbohydrates and their derivatives, polyamino acids or synthetic polymers.

8. A sphere or particle as claimed in claim 7, c h a r a c t e r i s e d in that the matrix, when it is a synthetic polymer, has been stabilised by means of covalent bonds.

9. A sphere or particle as claimed in one or more of claims 1-8, c h a r a c t e r i s e d in that its diameter is in the range of 0.01-1000 μm, preferably below 1 μm.

10. A sphere or particle as claimed in one or more of claims 1-9, c h a r a c t e r i s e d in that the particle per se has been built up of conglomerates of smaller particles or spheres.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE84/00437

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC 4

A 61 K 49/00, 49/02, 49/04

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
IPC 1	A 61 k 27/04, 27/08
IPC 2	A 61 K 29/00,02, 43/00
IPC 3-4	A 61 K 43/00, 49/00,02,04, 9/14,26 .../...

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [6]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	SE, B, 372 421 (MINNESOTA MINING AND MANUFACTURING COMPANY) 23 December 1974	1-10
X	SE, B, 7902009-5 (NEW ENGLAND NUCLEAR CORP) 3 January 1984	1-10
X	SE, B, 7407461-8 (PHARMACIA AB) 19 October 1981 See claims, page 2-3	1-10
X	SE, B, 7407462-6 (PHARMACIA AB) 19 October 1981 See claims, page 2-3	1-10
X	SE, B, 7607673-6 (THE PROCTER & GAMBLE COMPANY) 1 November 1982 See claims, page 3	1-10
X	EP, A2, 0 063 002 (RE SEARCH CORPORATION) 2 October 1982 & JP, 58013524 US, 4427646 AT, E, 11372 .../...	1-10

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1985-03-13	1985 -03- 26
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Solveig Eriksson Solveig Eriksson

VH

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

II

Fields searched (cont)

National Cl 30H:2/01, 10

US Cl 424:1, 1.1, 1.5

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ______, because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ______, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ______, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	US, A, 3 720 761 (WILLIAM W HUNTER) 13 March 1973 & NL, 6915452 DE, 1951769 FR, 2020684 GB, 1255670 CA, 949880	1-10
X	WO, A1, 78/00005 (KLAUS H MOSBACH) 7 December 1978 & JP, 54017110 EP, 0007932 GB, 2035798 SE, 7706431 SE, 431214	1-10
X	WO, A1, 83/01738 (ULF SCHRÖDER & KLAUS MOSBACH) 26 May 1983 & AU, 91274/82 EP, 0093757	1-10
X	DE, A1, 2 744 493 (JOHN WYETH & BROTHER LTD) 13 April 1978 See claims, page 7-9 & NL, 7710876 BE, 859291 FR, 2366835 LU, 78259 GB, 1548022 JP, 53044619 AU, 28825/77 CA, 1083042 AU, 513845 CH, 633717 US, 4371516 CA, 1097233 US, 4305502	1-10
A	WO, A1, 82/01006 (NATIONAL RESEARCH DEVELOPMENT CORPORATION) 1 April 1982	1-10
A	DE, C2, 2 336 546 (PHARMACIA AB) 31 January 1974	1-10
A	WO, A1, 82/00099 (KEY PHARMACEUTICALS INCORPORATED) 21 January 1982	1-10
P	WO, A1, 84/00294 (ULF SCHRÖDER) 2 February 1984	1-10
P	CH, A5, 641 682 (FELIX DAENIKER) 15 March 1984	1-10